What is claimed is:

CLAIMS

1. A microarray comprising a surface silanized with a silane in toluene in the absence of acetone or an alcohol, and a target molecule, wherein the target molecule is attached to the surface via the silane.

2. A microarray comprising a surface silanized with a silane in toluene in the absence of acetone or an alcohol, a linker, and a target molecule, wherein the target molecule is attached to the surface via the linker.

3. The microarray of claim 2, wherein the target molecule is a polynucleotide.

4. The microarray of claim 3, wherein the polynucleotide is selected from a group consisting of an oligonucleotide, DNA, amplified DNA, cDNA, single stranded DNA, double stranded DNA, PNA, RNA, and mRNA.

5. The microarray of claim 4, wherein the polynucleotide has a length in the range of about 3 bp to 10 kb.

6. The microarray of claim 5, wherein the length is in the range of about 100 bp to 5 kb.

7. The microarray of claim 6, wherein the length is in the range of about 0.3 kb to 3 kb.

8. The microarray of claim 7, wherein the length is in the range of about 0.5 kb to 2 kb.

9. The microarray of claim 4, wherein the polynucleotide is an oligonucleotide and the oligonucleotide is 25-1000 bp, 25-500, 30-200, and 50-100 bp in length.

10. The microarray of claim 2, wherein the target molecule is a polynucleotide and comprises an amine.

11. The microarray of claim 10, wherein the amine group is a primary amine.

12. The microarray of claim 11, wherein the primary amine is at the 5' end of the polynucleotide.

13. The microarray of claim 11, wherein the primary amine is attached at the 5' end of the polynucleotide via a linker, wherein the linker comprises one or more monomers of 1-20 carbon atoms, and wherein the monomer comprises a linear chain of carbons or a ring or both.

14. The microarray of claim 12, wherein the polynucleotide is prepared by extending a nucleic acid primer comprising a primary amine at its 5' end.

15. The microarray of claim 2, wherein the substrate surface is selected from the group consisting of polymeric materials, glasses, ceramics, natural fibers, nylon, nitrocellulose, silicons, metals, and composites thereof.

16. The microarray of claim 15, wherein the substrate surface is planar.

17. The microarray of claim 15, wherein the substrate is in a form of threads, sheets, films, gels, membranes, beads, plates, and like structures.

18. The microarray of claim 15, wherein the substrate surface is glass.

19. The microarray of claim 18, wherein the substrate is a glass slide.

20. The microarray of claim 2, wherein the target molecule is attached after contacting the target molecule with the surface by a technique selected from the group consisting of printing, capillary device contact printing, microfluidic channel printing, deposition on a mask, and electrochemical-based printing.

21. The microarray of claim 20, wherein the target molecule is unmodified prior to the contacting.

22. The microarray of claim 21, wherein the target molecule is modified to comprise an amine prior to the contacting.

23. The microarray of claim 22, wherein the amine is a primary amine.

24. The microarray of claim 23, wherein the target molecule is a polynucleotide and the primary amine is at the 5' end of the polynucleotide.

25. A microarray prepared by a method comprising:

(a) providing a multifunctional linker reagent comprising two or more reactive groups capable of reacting with a functional group on a surface of a microarray substrate and capable of reacting with a target molecule;

(b) activating the substrate surface for immobilizing the target molecule, by silanizing the surface with a silane in toluene in the absence of acetone or an alcohol, wherein the silane comprises a functionality reactive with the multifunctional linker reagent, and wherein the activating

further comprises immobilizing the multifunctional linker reagent on the silanized surface by attaching the multifunctional linker reagent to the silane via a first reactive group of the linker reagent and a reactive group of the silane;

(c) providing a solution comprising a target molecule having one or more functional groups reactive with a second reactive group of the immobilized multifunctional linker reagent;

(d) attaching the target molecule to the substrate surface by contacting the target molecule with the activated substrate surface under conditions that promote attachment of the target molecule to the immobilized multifunctional linker reagent.

26. The microarray of claim 25, wherein the target molecule is a polynucleotide, and wherein the contacting of step (d) is carried out by spotting the polynucleotide on an activated substrate surface.

27. The microarray of claim 26, wherein the polynucleotide is unmodified.

28. The microarray of claim 26, wherein the polynucleotide is modified with an amine group.

29. The microarray of claim 28, wherein the amine group is a primary amine at the 5' end of the polynucleotide.

30. The microarray of claim 26, wherein the polynucleotide is spotted on the surface at a concentration in the range of approximately 0.1 μg/μl to and including approximately 3 μg/μl.

31. The microarray of claim 25, wherein the attaching of step (d) occurs in a pH range from pH 6 to and including pH 10.

32. The microarray of claim 31, wherein the pH range is from pH 6.5 to and including pH 9.7.

33. The microarray of claim 32, wherein the pH range is from pH 7 to and including pH 9.4.

34. The microarray of claim 33, wherein the pH is 9.3.

35. The microarray of claim 25, wherein the attaching is allowed to occur for a time period from 1 minute to and including 24 hours.

36. The microarray of claim 35, wherein the time period is from 1 - 24 hours.

37. The microarray of claim 36, wherein the time period is from 5-18 hours.

38. The microarray of claim 37, wherein the time period is from 10-16 hours.

39. The microarray of claim 38, wherein the time period is from 12-14 hours.

40. The microarray of claim 25, wherein the method of preparing the microarray further comprises, after step (d), blocking unreacted reactive groups.

41. An activated slide comprising a substrate surface comprising a silane attached thereto, wherein the silanizing was in toluene, in the absence of acetone or an alcohol, and wherein the attached silane comprises at least one reactive functionality that is capable of reacting with a compound to immobilize the compound on the substrate surface.

42. The activated slide of claim 41, wherein the compound is selected from the group consisting of a modified target molecule, an unmodified target molecule, and a multifunctional linker reagent.

43. The activated slide of claim 42, wherein the compound is a multifunctional linker reagent comprising at least one reactive group capable of reacting with a target molecule to immobilize the target molecule on the substrate.

44. The activated slide of claim 42, wherein the target molecule is an unmodified polynucleotide comprising a native reactive group capable of reacting with the reactive functionality of the silane.

45. The activated slide of claim 43, wherein the target molecule is an unmodified polynucleotide comprising a native reactive group capable of reacting with the reactive group of the multifunctional linker reagent.

46. The activated slide of claim 43, wherein the target molecule is a modified polynucleotide comprising a non-native reactive group capable of reacting with the reactive group of the multifunctional linker reagent.

47. The activated slide of claim 46, wherein the target molecule is a polynucleotide and the non-native reactive group is an amine.

48. The activated slide of claim 47, wherein the amine is a primary amine.

49. The activated slide of claim 48, wherein the primary amine is at the 5' end of the polynucleotide.

50. The activated slide of claim 41, wherein the silane is an alkyl silane and the alkyl moiety is selected from the group consisting of an ethyl-, a propyl-, a butyl-, a pentyl-, a hexyl-, a heptyl-, an octyl-, a nonyl-, and a decylalkyl moiety, and the reactive functionality of the silane is selected from the group consisting of an amine, a hydroxyl moiety, an epoxide, a thiol, and a halide, and the reactive functionality is covalently linked to the alkyl moiety.

51. The activated slide of claim 50, wherein the reactive functionality of the silane is a primary amine on the alkyl moiety, and wherein at least one reactive group of the multifunctional linker reagent is a thiocyanate moiety, and wherein the multifunctional linker reagent is immobilized by covalent reaction with the primary amine of the silane of the silanized surface.

52. A method of activating a glass slide for immobilizing a target molecule, the method comprising silanizing the slide with a silane in toluene in the absence of acetone or an alcohol, wherein the silane is an alkyl silane and the alkyl moiety is selected from the group consisting of an ethyl-, a propyl-, a butyl-, a pentyl-, a hexyl-, a heptyl-, an octyl-, a nonyl-, and a decylalkyl moiety, and the reactive functionality of the silane is selected from the group consisting of an amine, a hydroxyl moiety, an epoxide, a thiol, and a halide, and the reactive functionality is covalently linked to the alkyl moiety.

53. The method of claim 52 further comprising reacting the silane with a multifunctional linker reagent comprising at least one reactive group capable of reacting with the silane and at least one reactive group capable of reacting with the target molecule for immobilizing the target molecule, wherein the reactive functionality of the silane is a primary amine on the alkyl moiety, and wherein at least one reactive group of the multifunctional linker reagent is a thiocyanate moiety, and wherein the multifunctional linker reagent is immobilized by covalent reaction with the primary amine of the silane of the silanized surface.

54. The method of claim 52, wherein the silane is an alkyl silane and the alkyl moiety is selected from the group consisting of an ethyl-, a propyl-, a butyl-, a pentyl-, a hexyl-, a heptyl-, an octyl-, a nonyl-, and a decylalkyl moiety, and the reactive functionality of the silane is selected from the group consisting of an amine, a hydroxyl moiety, an epoxide, a thiol, and a halide, and the reactive functionality is covalently linked to the alkyl moiety.

55. A method of preparing a microarray, the method comprising:
(a) providing an activated slide comprising a substrate surface comprising a silane attached thereto, wherein the silanizing was in toluene, in the absence of acetone or an alcohol, and wherein the attached silane

comprises at least one reactive functionality that is capable of reacting to immobilize a target molecule on the substrate surface;

(b) reacting the activated slide surface with the target molecule under conditions to immobilize the target molecule, wherein the target molecule is selected from the group consisting of a nucleic acid, a polynucleotide, RNA, single stranded DNA, double stranded DNA, an oligonucleotide, a peptide nucleic acid (PNA), a polypeptide, a protein, an antibody, a receptor, and a ligand.

56. The method of claim 55, further comprising after step (a) reacting the activated slide surface with a multifunctional linker reagent comprising at least two reactive groups capable of reacting with the silane to immobilize the multifunctional linker reagent on the surface, wherein the activated surface comprises the multifunctional linker reagent capable of reacting with the target molecule to immobilize the target molecule on the surface.

57. The method of claim 55, wherein the target molecule is a nucleic acid, a polynucleotide, a RNA, a single stranded DNA, a double stranded DNA, an oligonucleotide, or a peptide nucleic acid.

58. The method of claim 56, wherein the target molecule is a nucleic acid, a polynucleotide, a RNA, a single stranded DNA, a double stranded DNA, an oligonucleotide, or a peptide nucleic acid, and the multifunctional linker reagent reactive group is an isothiocyanate and the linker comprises from 1 to 20 carbon atoms.

59. The method of claim 58, wherein the multifunctional linker reagent comprises a plurality of linker monomers.

60. The method of claim 55, wherein the target molecule comprises is unmodified.

61. The method of claim 56, wherein the target molecule is modified and comprises an amine.

62. The method of claim 61, wherein the amine is a primary amine at the 5' end of the target molecule.

63. The method of claim 55, wherein the silane is 3-aminoproyltriethoxysilane.

64. The method of claim 56, wherein the multifunctional linker reagent is 1,4-phenylene diisothiocyanate.

65. A method of preparing a detectably labeled sDNA probe capable of forming a detectable complex with a target molecule immobilized on a microarray surface, the method comprising:

(a) isolating an amount of total cellular RNA from a biological sample;

(b) synthesizing a mixture of detectably labeled sDNA probes, wherein the synthesis of sDNA comprises synthesizing first strand cDNA from the isolated RNA of step (a), synthesizing second strand cDNA using Klenow fragment of DNA polymerase I and the first strand cDNA as template, synthesizing cRNA using the double stranded cDNA as template; and synthesizing sDNA using reverse transcriptase in the presence of detectably labeled deoxyribonucleotide using the cRNA as a template;

(c) isolating the labeled sDNA probes.

66. The method of claim 65, wherein the amount of total cellular RNA comprises from 0.01 to 10 pg messenger RNA.

67. The method of claim 66, wherein the amount of total cellular RNA is from 1-5 pg.

68. The method of claim 67, wherein the amount of total cellular RNA is from .5-2 pg.

69. The method of claim 65, wherein the synthesizing of sDNA is also in the presence of hexamer primers under conditions that cause the sDNA probes to have an average length of 0.5 - 2 kb.

70. A method of preparing a detectably labeled cDNA probe capable of forming a detectable complex with a target molecule immobilized on a microarray surface, the method comprising:

(a) isolating an amount of total cellular RNA from a biological sample;

(b) synthesizing a mixture of detectably labeled cDNA probes, wherein the synthesis of cDNA comprises synthesizing first strand cDNA from the isolated RNA of step (a) in the presence of detectably labeled deoxynucleotide;

(c) isolating the labeled sDNA probes.

71. A method of preparing a detectably labeled sDNA probe capable of forming a detectable complex with a target molecule immobilized on a microarray surface, the method comprising:

(a) isolating an amount of total cellular RNA from a biological sample;

(b) synthesizing a mixture of detectably labeled sDNA probes, wherein the synthesis of sDNA comprises synthesizing a biotin-attached first strand cDNA from the isolated RNA of step (a); synthesizing second strand DNA (sDNA) using Klenow fragment of DNA polymerase I and the first strand cDNA as template in the presence of detectably labeled deoxynucleotides;

(c) contacting the biotin-attached first strand cDNA with streptavidin and removing the biotin-first strand cDNA/streptavidn complex from the labeled sDNA; and

(c) isolating the labeled sDNA probes.

72. A method of preparing a detectably labeled cRNA probe capable of forming a detectable complex with a target molecule immobilized on a microarray surface, the method comprising:
(a) isolating an amount of total cellular RNA from a biological sample;
(b) synthesizing a mixture of detectably labeled cRNA probes, wherein the synthesis of cRNA comprises synthesizing first strand cDNA from the isolated RNA of step (a), synthesizing second strand cDNA using Klenow fragment of DNA polymerase I and the first strand cDNA as template, synthesizing cRNA using the double stranded cDNA as template in the presence of detectably labeled ribonucleotides; and
(c) isolating the labeled sDNA probes.

73. The method of claim 72, further comprising after step (c) degrading the cRNA probe with RNase under conditions such that the average length of the cRNA probe is adjusted to be from 0.5 kb to 3 kb.

74. The method of claim 71, wherein the step of synthesizing second strand DNA is in the presence of hexamer primers under conditions such that the average length of the labeled sDNA probe is from approximately 0.5 kb to approximately 2 kb.

75. The method of claim 70, further comprising after step (b) decreasing the average length of the labeled cDNA probes to be from 0.5 kb to 2 kb.

76. The method of claim 75, wherein the decreasing is by limited DNase digestion.

77. The method of claim 65, wherein the biological sample is selected from the group consisting of a cell, a tissue sample, a body fluid sample, and a mixture of synthetic oligonucleotides.

78. The method of claim 65, wherein the amount of total cellular RNA is from 0.5 pg to and including 10 mg.

79. The method of claim 78, wherein the amount of total cellular RNA is from 1 pg to and including 10µg.

80. The method of claim 79, wherein the amount of total cellular RNA is from 1 pg to and including 100 ng.

81. The method of claim 80, wherein the amount of total cellular RNA is from 1 pg to and including 10ng.

82. The method of claim 65, wherein the detectably labeled deoxynucleotide is labeled dUTP and the synthesizing in the presence of labeled dUTP is in the absence of unlabeled dTTP.

83. The method of claim 65, wherein the detectable label is a fluorochromophore.

84. A method of analyzing a target molecule attached to a microarray, the method comprising:
    (a) providing a microarray according to claim 1;
    (b) contacting the attached target molecule with an agent capable of forming a detectable complex with the target molecule under conditions that allow formation of a detectable complex;
    (c) detecting formation of a detectable complex;
    (d) determining the amount of a detectable complex formed.

85. The method of claim 84, wherein the agent capable of forming a detectable complex comprises:
    a control mixture of sDNA probes comprising a first detectable label, wherein the probes are prepared from total cellular RNA isolated from a control sample, and
    a test mixture of sDNA probes comprising a second detectable label, wherein the probes are prepared from total cellular RNA isolated from a test sample,
    wherein the first and second detectable labels are distinguishable,
    wherein the method further comprises:
    (1) pooling the control sDNA probes and the test sDNA probes;
    (2) performing steps (a) – (d) of claim 84; and
    (3) comparing the amount of detectable complex formed between the target molecule and the control probes relative to the amount of complex formed between the target molecule and the test probes.

86. The method of claim 84, wherein the label is optically detectable.

87. The method of claim 86, wherein the label is fluorescent.

88. The method of claim 84, wherein the contacting of step (b) occurs in the absence of detergent.

89. The method of claim 88, wherein the contacting of step (b) occurs in the presence of formamide and a one or more of dimethylsulfoxide (DMSO), tetramethylammonium chloride (TMACl), and tetraethylammonium chloride (TEACl).

90. The method of claim 89, wherein the contacting of step (b) occurs in the presence of formamide, DMSO and TMACl or TEACl, wherein the sum of the proportions of formamide and DMSO does not exceed 50%.

91. The method of claim 90, wherein the sum of the proportions of formamide and DMSO does not exceed 25%.

92. The method of claim 88, further comprising a wash step subsequent to the contacting step wherein the wash solution comprises detergent.

93. A method of hybridizing a detectable polynucleotide probe to a target polynucleotide on a support surface, the method comprising:

(a) contacting the probe with denatured target polynucleotide on the support surface in a hybridization solution comprising DMSO or formamide or both, and in the absence of detergent; and

(b) detecting formation of a complex between the target polynucleotide and the detectably labeled polynucleotide probe.

94. The method of claim 93, wherein the sum of the proportions of DMSO and formamide does not exceed 50%, and wherein the hybridization solution further comprises TMACl or TMECl or both.

95. The method of claim 94, wherein the sum of the proportions of DMSO and formamide does not exceed 25%, and wherein the hybridization solution further comprises TMACl or TMECl or both.

96. The method of claim 85, wherein the control sample comprises cells removed from a cell source by laser capture microdissection, wherein the cell source is selected from the group consisting of untreated tissue, frozen tissue, paraffin-embedded tissue, stained tissue, and cell culture.

97. The method of claim 85, wherein the test sample comprises cells removed from a cell source by laser capture microdissection, wherein the cell source is selected from the group consisting of untreated tissue, frozen tissue, paraffin-embedded tissue, stained tissue, and cell culture.

98. 98. The method of claim 85, wherein the test sample and control sample differ according to one or more of developmental state, disease state, pre-disease state, cell type, sample source, and experimental treatment conditions.

99. The method of claim 85, wherein the target molecule is a polynucleotide and the nucleic acid isolated from the test sample and the control sample is RNA, and wherein the comparing of step (c) provides a measure of target polynucleotide expression in the test sample relative to target polynucleotide expression in the control sample.

100. The method of claim 99, wherein the relative measure of target polynucleotide expression indicates a disease state in the test tissue sample.

101. The method of claim 100, wherein the disease state is selected from the group consisting of tumor, cardiovasular disease, inflammatory disease, endocrine disease.

102. The method of claim 100, wherein the relative measure of target polynucleotide expression indicates a pre-disease state in the test tissue sample.

103. The method of claim 84, wherein the target molecule is a polynucleotide and the nucleic acid isolated from the test sample and the control sample is DNA, and wherein the comparing of step (c) provides a measure of number of copies of the target polynucleotide in cells of the test sample relative to target polynucleotide copies in the control sample.

104. The method of claim 103, wherein the relative measure of the number of copies of target polynucleotide indicates a disease state or a pre-disease state in the test tissue sample.